(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 699 761 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
06.03.1996 Bulletin 1996/10

(51) Int. Cl.[6]: **C12N 15/54**, C12N 9/10

(21) Application number: **95111764.7**

(22) Date of filing: **26.07.1995**

(84) Designated Contracting States:
BE CH DE FR GB IT LI SE

(30) Priority: **29.07.1994 JP 179336/94**

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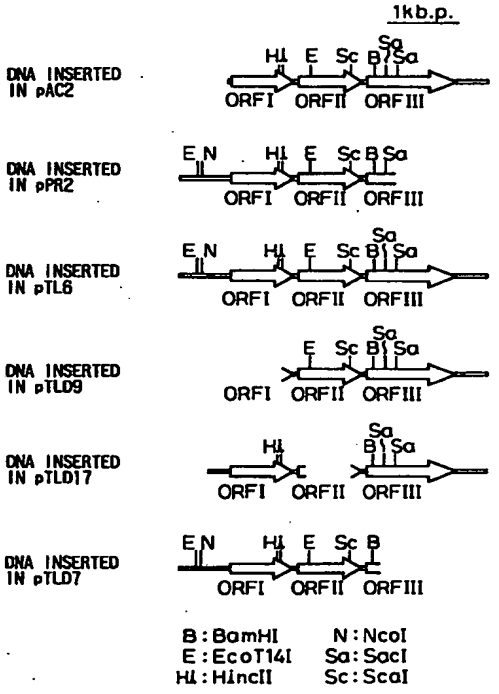
Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Heptaprenyl diphosphate synthesizing enzyme and DNA encoding same

(57) Heptaprenyl diphosphate (HDP)-synthetase derived from *Bacillus stearothermophilus* which enzymes have the amino acid sequences shown as SEQ ID NOs: 1 to 3; 1 and 2; 2 and 3; or 1 and 3, DNA encoding them, and a method of producing the enzymes.

According to the invention it is possible to industrially produce HDP-synthesizing enzyme and HPD.

Fig.1



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Description

BACKGROUND OF INVENTION

1. Field of Invention

The present invention relates to heptaprenyl diphosphate (hereunder sometimes abbreviated to "HDP") synthetase of *Bacillus stearothermophilus* origin, to DNA encoding the enzyme, to an expression vector containing the DNA, to a host transformed by the expression vector, to a method of producing heptaprenyl diphosphate-synthesizing enzyme by the host, and to a method of producing heptaprenyl diphosphate using the enzyme or host.

2. Related Art

HDP, synthesized from condensation reaction of 4 molecules of isopentenyl diphosphate and 1 molecule of farnesyl diphosphate by HDP-synthetase, is an important biosynthetic intermediate of isoprenoids such as prenylquinone. Although HDP-synthetase, which is categorized into prenyl transferase, is known to be present in some microorganisms such as *Bacillus subtilis* (J. Biol. Chem. 255, p.4539-4543 (1980)), its amino acid sequence and the DNA sequence of the gene encoding it have not been known.

Genes coding for other prenyl transferase are known, farnesyl diphosphate synthetase ([2.5.1.1.] J. Biol. Chem. 265, p.4607-4614 (1990)), geranylgeranyl diphosphate synthetase (Proc. Natl. Acad. Sci. USA, 89, p.6761-6764). However, the tertially structures of the known prenyl transferases are homodimers which comprise of two exactly same subunits, and it is different from the peculiar heterodimer of *Bacillus subtilis* HDP synthetase (FEBA Letl. 161, 257-260 (1983)). Therefore, absolutely no data exists regarding homology between the amino acid sequences of the former two and the latter.

Consequently, the present invention is aimed at providing HDP synthetase of *Bacillus stearothermophilus* origin, which was hitherto unknown in the species, DNA encoding the enzyme, and a method of production of the recombinant HDP synthetase using the DNA.

SUMMARY OF INVENTION

With the aim of accomplishing the above-mentioned object, the present inventors have been the first to succeed in cloning an HDP synthetase gene of *Bacillus stearothermophilus* origin, by the PCR method using synthesized primers designed from a portion of the known sequence of prenyl transferase, following hybridization using PCR amplified fragments as probe and measuring the expressed activity of the gene expression products.

Thus, the present invention provides a protein of *Bacillus stearothermophilus* origin having heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; a peptide with the amino acid sequence from the 1st amino acid Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention also provides a peptide of *Bacillus stearothermophilus* origin, which has the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention further provides a peptide of *Bacillus stearothermophilus* origin, which has the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention further provides a protein of *Bacillus stearothermophilus* origin with heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention further provides a protein of *Bacillus stearothermophilus* origin with heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid

Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention further provides a protein of *Bacillus stearothermophilus* origin with heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

The present invention further provides DNA encoding the above-mentioned protein and various peptides.

The present invention further provides an expression vector comprising the above-mentioned DNA.

The present invention further provides a host transformed by the above-mentioned expression vector.

The present invention further provides a method of producing heptaprenyl diphosphate synthetase which is characterized by culturing the above-mentioned host, and collecting heptaprenyl diphosphate synthetase from the cultured product.

The present invention further provides a method of producing heptaprenyl diphosphate which is characterized by culturing the above-mentioned transformant, and collecting heptaprenyl diphosphate from the cultured product.

The present invention further provides a method of producing heptaprenyl diphosphate which is characterized by reacting the above-mentioned enzyme with a substrate.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the positional relationships and restriction enzyme maps for plasmids pAC2, pPR2, pTL6, pTLD9, pTLD17 and pTLD7 of the present invention.

Fig. 2 is a thin layer radiochromatograms of the reaction mixture prepared by incubation of isopentenyl diphospate and farnesyl diphosphate with expression product of a DNA fragment of the present invention.

DETAILED DESCRIPTION

The open reading frame portions of nucleotide sequences of DNA cloned from *Bacillus stearothermophilus* which express heptaprenyl diphosphate synthetase activity are shown as SEQ ID NOs: 1 to 3. There are 3 open reading frames (ORF). The first open reading frame (ORFI) is assumed to begin at the ATG coding for the 1st amino acid Met of SEQ ID NO: 1 and to end with the GGG coding for the 220th Gly. However, it may possibly begin at the ATG coding for the 19th amino acid Met, the ATG coding for the 20th amino acid Met, or the ATG coding for the 22nd amino acid Met.

The second open reading frame (ORFII) is assumed to begin at the ATG coding for the 1st amino acid Met of SEQ ID NO: 2 and to end with the CGG coding for the 234th amino acid Arg. However, this ORFII may possibly begin at the ATG coding for the 23rd amino acid Met of the amino acid sequence. The third open reading frame (ORFIII) is assumed to begin at the GTG coding for the 1st amino acid Val of SEQ ID NO: 3, and to end with the TAT coding for the 323rd amino acid Tyr. However, this ORFIII may possibly begin at the ATG coding for the 4th amino acid Met or the ATG coding for the 9th amino acid Met.

In the DNA containing the cloned ORFI-III, the nucleotide AACG locates between the translation termination codon TAG at the 3' end of ORFI and the translation initiation codon ATG (Met) of ORFII, and the nucleotide GTTAAG locates between the translation termination codon TGA of ORFII and the translation initiation codon GTG (Val) of ORFIII.

The full-length DNA expression product had the strongest heptaprenyl diphosphate synthetase activity and the expression products of ORFI and ORFIII, ORFI and ORFII, and ORFII and ORFIII also showed heptaprenyl diphosphate synthetase activity. Consequently, according to one embodiment of the present invention, there are provided DNA comprising all of ORFI, ORFII and ORFIII, heptaprenyl diphosphate synthetase consisting of the peptide encoded thereby, and a method for its production.

The present invention also provides DNA containing ORFI and ORFIII but not containing ORFII in its complete form, a peptide having heptaprenyl diphosphate synthetase activity which is expressed by that DNA, and a method for its production. The present invention further provides DNA containing ORFI and ORFII, or ORFII and ORFIII but not containing any other ORF in its complete form, a peptide expressed thereby, and a method for its production.

Plant-derived enzymes sometimes differ in a few amino acids depending on the variety of plants from which they are derived, and often differ in a few amino acids by natural mutations. In addition, the native activity of an enzyme is sometimes maintained even upon artificial mutation on the amino acid sequence. Consequently, the present invention also encompasses, in addition to peptides having the amino acid sequences represented by SEQ ID NOs: 1 to 3, also peptides with amino acid sequences resulting from variations of the amino acid sequences represented by SEQ ID NOs: 1 to 3 by means of a substitution, deletion and/or addition of one or a few, for example 5 or 10, amino acids, providing that the peptides are still have the enzyme activity.

The present invention further provides DNA encoding a peptide mutated in the manner described above, as well as a method of producing the mutated peptide.

As will be explained in detail by way of the examples, the DNA of the present invention may be cloned from *Bacillus stearothermophilus*. Also, DNA containing any one of ORFI, ORFII and ORFIII, all three, or ORFI and ORFIII, ORFI and ORFII or ORFII and ORFIII, and not containing any other ORF in its complete form, may be obtained by cutting full-length DNA using restriction endonucleases which cut within, for example, other ORFs outside of the aimed ORF without cutting within the latter. Alternatively, DNA encoding a mutated peptide may be obtained by the site-specific mutagenesis using, for example, a mutagenic primer.

Furthermore, once the amino acid sequence of one peptide is determined, it is possible to define a proper nucleotide sequence coding therefor, which then allows chemical synthesis of the DNA by conventional DNA synthesis methods. Each individual ORF of the present invention is not especially long, and thus may be easily synthesized by a person skilled in the art by conventional DNA synthesis methods.

The present invention further provides expression vectors comprising the DNA as described above, hosts transformed by the expression vectors, and a method of producing the enzyme or peptides of the present invention using these hosts.

The expression vector includes an origin of replication, the expression regulating sequence, etc., which differ depending on the host. The host may be a prokaryotic organism, for example a bacterium such as an *E. coli*, or *Bacillus* such as *Bacillus subtilis*; a eukaryotic organism, for example yeast, a fungus an example of which is *S. cerevisiae* belonging to the genus *Saccharomyces*, or fungus an example of which is a mold such as *A. niger* or *A. oryzae* belonging to the genus *Aspergillus*; animal cells such as cultured silk worm cells or cultured higher animal cells, for example CHO cells. Plant cells may also be used as hosts.

According to the present invention, as will be shown in the examples, it is possible to produce heptaprenyl diphosphate synthase by culturing a host transformed with DNA of the present invention, which accumulates the enzyme in the culture, and recovering it. Also, according to the present invention, heptaprenyl diphosphate may also be produced by allowing HDP synthetase produced by the method of the present invention to react with isopentenyl diphosphate and allylic diphosphate such as farnesyl diphosphate acid as substrates.

Referring to the use of *E. coli* as a host for an example, there are known gene expression regulating mechanism in the process of transcription of mRNA from DNA, the process of translation of protein from mRNA, etc. As promoter sequences which regulate mRNA synthesis, there are known, in addition to naturally occurring sequences (for example, lac, trp, bla, lpp, P_L, P_R, ter, T3, T7, etc.), also mutants thereof (for example, lacUV5) and sequences obtained by artificially fusing natural promoter sequences (for example, tac, trc, etc.), and these may also be used according to the present invention.

As sequences capable of regulating ability to synthesize protein from mRNA, the importance of the ribosome-binding site (GAGG and similar sequences) and the distance to the initiation codon ATG is already known. It is also well known that terminator sequences which govern completion of transcription at the 3' end (for example, vectors including $rrnBT_1T_2$ are commercially available from Pharmacia Co.) affect the efficiency of protein synthesis in recombinants.

Vectors which may be used to prepare the recombinant vectors of the present invention may be commercially available ones, or they may be any of a variety of derived vectors, depending on the purpose. As examples there may be mentioned pBR322, pBR327, pKK223-3, pKK233-2, pTrc99, etc. which carry the pMB1-derived replicon; pUC18, pUC19, pUC118, pUC119, pHSG298, pHSG396, etc. which have been modified for increased number of copies; pACYC177, pACYC184, etc. which carry the p15A-derived replicon; and plasmids derived from pSC101, ColE1, R1 or F factor.

In addition to plasmids, gene introduction is also possible by way of virus vectors such as λ-phage and M13 phage, and transposons. For gene introduction to microorganisms other than *E. coli*, there is known gene introduction to the genus *Bacillus* by pUB110 (available from Sigma Co.) and pHY300PLK (available from Takara Shuzo). These vectors are described in Molecular Cloning (J. Sambrook, E.F. Fritsch, T. Maniatis, published by Cold Spring Harbor Laboratory Press), Cloning Vector (P.H. Pouwels, B.E. Enger/Valk, W.J. Brammar, published by Elsevier), and various company catalogs.

In particular, pTrc99 (available from Pharmacia Co.) is preferred as a vector including, in addition to the ampicillin resistance gene as a selective marker, Ptrc and $lacI^q$ as a promoter and controlling gene, the sequence AGGA as a ribosome-binding site, and $rrnBT_1T_2$ as the terminator, and having an expression regulating function on the HDP-synthesizing enzyme gene.

The incorporation into these vectors of a DNA fragment coding for HDP synthetase and if necessary a DNA fragment with the function of expression regulation on the gene for the above-mentioned enzyme, may be accomplished by a known method using an appropriate restriction endonuclease and ligase. Specifically the method described below may be conveniently followed. pTL6 may be mentioned as a definite plasmid of the present invention prepared in this manner.

As microorganisms for the gene introduction by such recombinant vectors, there may be used *Escherichia coli*, as well as microorganisms belonging to the genus *Bacillus*. The transformation may also be carried out by a conventional method, for example the $CaCl_2$ method or protoplast method described in Molecular Cloning (J. Sambrook, E.F. Fritsch,

T. Maniatis, published by Cold Spring Harbor Laboratory Press) or DNA Cloning Vol.I-III (ed. by D.M. Glover, published by IRL PRESS), etc.

A representative transformant according to the present invention which may be obtained is pTL6/JM109.

When these transformants or recombinant microorganism cells are cultured in medium normally used for *E. coli*, heptaprenyl diphosphate synthase (HDP synthase) accumulates in the cells. The HDP in the cells may be recovered by physical treatment in the absence or presence of a cytolytic enzyme for lysis and a conventional isolation and purification method of for enzymes.

Lysozyme is preferably used as the cytolytic enzyme, and ultrasonic waves are preferably used for physical treatment. Most of the *E. coli*-derived protein may be removed as insoluble deposit by heating at about 55°C. For the isolation and purification of the enzyme, any or a combination of gel filtration, ion exchange, hydrophobic, reverse phase, affinity or other type of chromatography, or ultrafiltration may be available.

During the process of isolation and purification, a reagent to stabilize the desired enzyme may be combined with the treatment solution, for example, a reducing agent such as β-mercaptoethanol or dithiothreitol, protective agent against proteases, such as PMSF or BSA, or metal ion such as magnesium.

Since the above-mentioned HDP synthetase activity may be measured, for example, in the manner described hereunder, it is recommended that the isolation and purification of the enzyme be performed while confirming the activity of the enzyme using the assay reaction solution employed in f) in Example 1 hereunder.

EXAMPLES

An example of a method of preparing a DNA sequence, plasmid and transformant according to the present invention will now be described, but the scope of the invention is in no way restricted to this example.

Example 1

The experiment was carried out basically in accordance with Molecular Cloning, DNA Cloning and the Takara Shuzo Catalog, mentioned previously. Most of the enzymes used were purchased from Takara Shuzo. The *Bacillus stearothermophilus* used was the known bacterium stored at the American Type Culture Collection (ATCC). Strain ATCC 10149 was used for this experiment.

a) Preparation of chromosomal DNA of *Bacillus stearothermophilus*

Culturing was performed in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl) at 55°C, and the cells were collected. After suspension in a lysis buffer, lysozyme (chicken albumen-derived, product of Sigma Co.) was added to 10 mg/ml. After lysis, 1/10 volume of 1M Tris·HCl (pH 8.0), 1/10 volume of 10% SDS and 1/50 volume of 5 M NaCl were added. Proteinase K (product of Sigma Co.) was added to 10 mg/ml, and the mixture was heated to 50°C.

An equivalent of phenol was added and the mixture stirred and centrifuged to remove the protein. The supernatant was taken with a wide-mouthed pipette into a beaker, and after a 2.5-fold amount of ethanol was gently layered thereon the chromosomal DNA was wound up on a glass rod. After dissolution in TE (10 mM Tris·HCl (pH 8.0), 1 mM EDTA), the DNA was treated with RNaseA (product of Sigma Co.), Proteinase K and phenol, a 2.5-fold amount of ethanol was gently layered thereon and the chromosomal DNA was wound up on a glass rod. After washing with 70% ethanol, it was dissolved in TE and used in the following experiment.

b) Acquisition of pCR64

DNA primers P1 (Sequence No. 4), P2 (Sequence No. 5), P4 (Sequence No. 6), P6 (Sequence No. 7), P8 (Sequence No. 8), P9 (Sequence No. 9), P10 (Sequence No. 10), P11 (Sequence No. 11), P12 (Sequence No. 12) and P13 (Sequence No. 13) were prepared based on the heretofore known conserved regions of the amino acid sequence of prenyl transferase.

The chromosomal DNA was subjected to partial digestion with Sau3AI, and the PCR (polymerase chain reaction) was conducted with combinations of synthetic DNA P1 and P4, P1 and P6, P1 and P8, P2 and P4, P2 and P6, P2 and P8, P9 and P11, P9 and P4, P9 and P6, P9 and P8, P9 and P13, P1 and P11, P2 and P11, P12 and P4, P12 and P6, P12 and P8, P12 and P13, P1 and P13, P2 and P13, P10 and P4, P10 and p6, P10 and P8, and P10 and P13.

The PCR product of the P10 and P8 combination was linked with the HincII digestion product of plasmid pUC118 (purchased from Takara Shuzo) using T4DNA ligase, and *E. coli* JM109 was transformed. Plasmids were prepared by the alkali SDS method, and the DNA sequences of 27 clones were analyzed with an Applied Biosystems 373A fluorescent

DNA sequencer. One of the sequences was referred as pCR64.

Table 1

(Composition of PCR reaction solution)

Template DNA	1 µg
10 x Amplitaq Buffer	10 µl
dNTPs mixture solution (1.25 mM each)	16 µl
Primer 1	100 pmol
Primer 2	100 pmol
Taq polymerase adjusted to 100 µl with H_2O	2 units

(PCR reaction conditions)

94°C, 30 secs
↓
50°C, 30 secs ┐
↓ ├ × 35 cycles
72°C, 1 min ┘
↓
72°C, 7 mins
↓
4°C

c) Cloning of surrounding region with pCR64 as probe

c-1) A DNA fragment consisting of an approximately 500 bp pCR64 digestion product by restriction endonucleases KpnI and HindIII was labelled with DIG using a DIG DNA labeling kit (purchased from BOEHRINGER MANNHEIM). The instructions in the kit manual were followed.

c-2) Preparation of library

The chromosomal DNA was digested with restriction endonuclease AccI, and upon Southern hybridization using the probe from c-1), a band was detected in the position of about 3 kbp. Here, the DNA fragment of about 3 kbp was isolated by agarose gel electrophoresis and treated with T4DNA polymerase. These were linked with the SmaI digestion product of plasmid pUC18 using T4DNA ligase, and *E. coli* JM109 was transformed.

c-3) Screening

The library prepared in c-2) was screened with the probe prepared in c-1). Detection was made using a DIG DNA detection kit (purchased from BOEHRINGER MANNHEIM) and plasmid pAC2 was obtained. The instructions in the kit manual were followed. DNA sequence of the inserted gene of about 2.5 kbp was analyzed with an Applied Biosystems 373A fluorescent sequencer.

d) Isolation of pPR2

The gene library of c-2) was subjected to PCR using a synthetic DNA primer P64-4 (Sequence No. 14) prepared based on the DNA sequence obtained in c-3) and M13 Primer RV (purchased from Takara Shuzo). The amplification product was inserted into pT7 Blue T-Vector (purchased from Novagen) to obtain pPR2.

e) Linking of pAC2 and pPR2

DNA fragments of about 1 kbp and 5 kbp as BamHI digestion products of pAC2 and pPR2, respectively, were ligated to obtain pTL6.

f) Measurement of isoprenoid synthetase activity

The *E. coli* JM105 transformed with pTL6 was cultured overnight in 50 ml of LB medium containing 50 µg/ml of ampicillin, and the cells were collected. These were suspended in 4 ml of lysis buffer and disrupted with ultrasonic waves. Heating was performed at 55°C for 1 hour to inactivate the *E. coli*-derived prenyl transferase, and the *E. coli*-derived

denatured protein was removed by centrifugation and the supernatant was used for the assay. The assay reaction mixture was allowed to react for 1 hour or 14 hours at 55°C. The reaction mixture was extracted with 1-butanol, and the radioactivity was measured using a liquid scintillation counter.

Table 2

(Composition of lysis buffer)	
Tris·HCl (pH 7.7)	50 mM
EDTA	1 mM
β-Mercaptoethanol	10 mM
PMSF	0.1 mM
(Composition of assay reaction solution (total volume: 1 ml))	
Tris·HCl (pH 8.5)	50 mM
$MgCl_2$	25 mM
NH_4Cl	50 mM
β-Mercaptoethanol	50 mM
(all-E)-farnesyl diphosphate	25 nmoles
[1-^{14}C]Isopentenyl diphosphate (product of Amersham Col., corresponding to approx. 5.5×10^4 dpm)	25 nmoles
Cell-free extract	500 µl

The 1-butanol extract obtained from the above-mentioned reaction of JM105 carrying pTL6 was hydrolyzed and analyzed by thin-layer chromatography (TLC). As a result, the produced isoprenoid was identified as heptaprenyl diphosphate, thus showing that pTL6 contains the gene for heptaprenyl diphosphate synthetase (Fig. 2). Furthermore, upon investigating the specificity to allylic substrate primers in the assay system described hereunder (Table 3), particular enzyme activity was found with (all-E) farnesyl diphosphate and (all-E) geranylgeranyl diphosphate, whereas dimethylallyl diphosphate, geranyl diphosphate, (2Z, 6E)-farnesyl diphosphate, (2Z, 6E, 10E) geranylgeranyl diphosphate and (2Z, 6E, 10E, 14E) farnesylgeranyl diphosphate were not satisfactory substrates (Table 4).

Table 3

(Composition of assay reaction solution (total volume: 1 ml))	
Tris·HCl (pH 8.5)	50 mM
$MgCl_2$	25 mM
NH_4Cl	50 mM
β-Mercaptoethanol	50 mM
Allylic substrate	2.5 nmoles
[1-^{14}C]Isopentenyl diphosphate (product of Amersham Col., corresponding to approx. 1.1×10^5 dpm)	0.92 nmoles
Cell-free extract	500 µl

Table 4

Substrate specificity of HDP synthetase derived from DNA sequence of the present invention	
Substrate	Enzyme activity (dpm)
Dimethylallyl diphosphate	324
Geranyl diphosphate	381
(all-E) Farnesyl diphosphate	4163
(2Z, 6E) Farnesyl diphosphate	323
(all-E) Geranylgeranyl diphosphate	1514
(2Z, 6E, 10E) Geranylgeranyl diphosphate	648
(all-E) Farnesylgeranyl diphosphate	728
(2Z, 6E, 10E, 14E) Farnesylgeranyl diphosphate	281

E. coli normally has no heptaprenyl transferase or prenyl transferase with activity at 55°C. *E. coli* transformed with pTL6 is able to synthesize heptaprenyl diphosphate. Also, the fact that the activity is present at 55°C indicates that the *Bacillus stearothermophilus*-derived prenyl transferase encoded by pTL6 is highly thermostable. This also shows that the recombinant is useful for producing stable heptaprenyl diphosphate.

g) Preparation of pTL6 deletion mutants and identification of HDP synthetase gene

pTL6 had a gene insert of about 3 kbp, which contained three ORFs. Upon cleavage of pTL6 with restriction endonuclease and preparation of plasmid pTLD9 by deletion of ORFI, plasmid pTLD17 by deletion of OFRII and plasmid pTLD7 by deletion of ORFIII, and measurement of the isoprenoid-synthetase activities, activity was found for pTL6, pTLD9 and pTLD17. 1-Butanol extracts of reaction products of pTL6 and pTLD17 were hydrolyzed and analyzed by TLC, and the produced isoprenoid was confirmed to be heptaprenyl diphosphate.

Table 5

HDP synthetase activities derived from DNA sequences of the present invention (Radioactivity of 1-butanol extracts expressed in dpm units)	
Cell-free extract solution	Enzyme activity (dpm)
E. coli JM105	0
E. coli JM105 / pT7Blue T-Vector	0
E. coli JM105 / pTL6	750
E. coli JM105 / pTLD9	16
E. coli JM105 / pTLD17	129(*)
E. coli JM105 / pTLD7	0

* = 14 hour reaction

According to the present invention there are provided DNA sequences coding for heptaprenyl diphosphate synthetase enzyme of *Bacillus stearothermophilus* origin. Recombinant microorganisms, obtained by incorporating the DNA sequences into expression vectors which are then used to transform appropriate *E. coli* strains, produce safe substances with heptaprenyl diphosphate synthetase activity and heptaprenyl diphosphate.

This effect is achieved by preparing the above-mentioned DNA sequences from chromosomes of *Bacillus stearothermophilus*, which is not so far taught in scientific literature.

SEQUENCE LISTINGS

Sequence No.: 1
Sequence length: 663
Sequence type: nucleic acid
Strandedness: double
Topology: linear
Molecule type: Genomic DNA
Original source:
Organism: *Bacillus stearothermophilus*
Sequence:

```
ATG CTC GAT GGC GCT TCA ACG GCG CCG AGT GAG GCG GAG CGG TGC ATC   45
Met Leu Asp Gly Ala Ser Thr Ala Pro Ser Glu Ala Glu Arg Cys Ile
                 5                  10                  15
ATC GCC ATG ATG CTC ATG CAG ATC GCC CTT GAT ACC CAC GAT GAG GTG   90
Ile Ala Met Met Leu Met Gln Ile Ala Leu Asp Thr His Asp Glu Val
            20                  25                  30
ACA GAT GAC GGC GGC GAC TTG CGG GCG CGG CAG CTT GTC GTC CTG GCC  135
Thr Asp Asp Gly Gly Asp Leu Arg Ala Arg Gln Leu Val Val Leu Ala
        35                  40                  45
GGC GAC TTG TAC AGC GGG CTG TAC TAT GAG TTG TTG GCG CGT TCG GGC  180
Gly Asp Leu Tyr Ser Gly Leu Tyr Tyr Glu Leu Leu Ala Arg Ser Gly
    50                  55                  60
GAA ACG GCG CTC ATC CGC TCG TTC GCC GAG GCG GTC CGC GAT ATT AAC  225
Glu Thr Ala Leu Ile Arg Ser Phe Ala Glu Ala Val Arg Asp Ile Asn
65                  70                  75                  80
GAG CAA AAA GTG CGG CTT TAC GAA AAA AAA GTA GAG CGG ATC GAG TCG  270
Glu Gln Lys Val Arg Leu Tyr Glu Lys Lys Val Glu Arg Ile Glu Ser
                85                  90                  95
TTG TTT GCG GCG GTC GGC ACG ATC GAA TCG GCG TTG CTT GTC AAG CTC  315
Leu Phe Ala Ala Val Gly Thr Ile Glu Ser Ala Leu Leu Val Lys Leu
            100                 105                 110
GCC GAC CGC ATG GCG GCG CCG CAG TGG GGG CAG TTT GCC TAT TCG TAT  360
Ala Asp Arg Met Ala Ala Pro Gln Trp Gly Gln Phe Ala Tyr Ser Tyr
        115                 120                 125
```

```
TTG CTG ATG CGG CGC CTG CTG CTC GAG CAG GAA GCG TTC ATC CGC ACG  405
Leu Leu Met Arg Arg Leu Leu Leu Glu Gln Glu Ala Phe Ile Arg Thr
    130                 135                 140
GGA GCT TCG GTG CTC TTT GAG CAA ATG GCG CAA ATC GCG TTC CCG CGC  450
Gly Ala Ser Val Leu Phe Glu Gln Met Ala Gln Ile Ala Phe Pro Arg
145                 150                 155                 160
GCG GAA ACG TTG ACG AAA GAG CAA AAG CGG CAT TTG CTC CGC TTT TGC  495
Ala Glu Thr Leu Thr Lys Glu Gln Lys Arg His Leu Leu Arg Phe Cys
                165                 170                 175
CGC CGC TAT ATC GAC GGC TGC CGG GAG GCG CTG TTT GCG GCG AAA CTG  540
Arg Arg Tyr Ile Asp Gly Cys Arg Glu Ala Leu Phe Ala Ala Lys Leu
            180                 185                 190
CCG GTC AAC GGC CTG CTG CAG CTC CGC GTG GCC GTG CTT TCC GGC GGG  585
Pro Val Asn Gly Leu Leu Gln Leu Arg Val Ala Val Leu Ser Gly Gly
        195                 200                 205
TTT CAA GCC ATC GCC AAA AAG ACG GTG GAA GAA GGG TAG              630
Phe Gln Ala Ile Ala Lys Lys Thr Val Glu Glu Gly ***
    210                 215                 220
                                                                 663
```

Sequence No.: 2
Sequence length: 705
Sequence type: nucleic acid
Strandedness: double
Topology: linear
Molecule type: Genomic DNA
Original source:
Organism: *Bacillus stearothermophilus*
Sequence:

```
ATG CGT CAA TCG AAA GAA GAG CGA GTC CAT CGC GTA TTT GAA AAC ATT   45
Met Arg Gln Ser Lys Glu Glu Arg Val His Arg Val Phe Glu Asn Ile
                5                   10                  15
TCT GCG CAT TAT GAC CGG ATG AAC TCC GTC ATC AGC TTC CGC CGC CAC   90
Ser Ala His Tyr Asp Arg Met Asn Ser Val Ile Ser Phe Arg Arg His
            20                  25                  30
```

```
TTG AAG TGG CGC AAA GAC GTG ATG CGG CGG ATG AAT GTG CAA AAA GGC  135
Leu Lys Trp Arg Lys Asp Val Met Arg Arg Met Asn Val Gln Lys Gly
          35                  40                  45
AAA AAA GCG CTC GAT GTG TGC TGT GGG ACG GCT GAC TGG ACG ATC GCC  180
Lys Lys Ala Leu Asp Val Cys Cys Gly Thr Ala Asp Trp Thr Ile Ala
     50                  55                  60
TTG GCG GAG GCG GTC GGT CCG GAA GGG AAA GTG TAC GGC CTT GAT TTC  225
Leu Ala Glu Ala Val Gly Pro Glu Gly Lys Val Tyr Gly Leu Asp Phe
 65                  70                  75                  80
AGC GAA AAC ATG CTG AAA GTC GGC GAA CAG AAG GTA AAA GCG CGC GGG  270
Ser Glu Asn Met Leu Lys Val Gly Glu Gln Lys Val Lys Ala Arg Gly
                 85                  90                  95
TTG CAT AAT GTG AAG CTC ATT CAC GGC AAT GCG ATG CAG CTG CCG TTT  315
Leu His Asn Val Lys Leu Ile His Gly Asn Ala Met Gln Leu Pro Phe
            100                 105                 110
CCT GAC AAT TCG TTC GAT TAT GTG ACG ATC GGC TTC GGT TTG CGC AAC  360
Pro Asp Asn Ser Phe Asp Tyr Val Thr Ile Gly Phe Gly Leu Arg Asn
        115                 120                 125
GTC CCT GAC TAT ATG ACC GTG CTT AAG GAA ATG CAC CGG GTG ACG AAG  405
Val Pro Asp Tyr Met Thr Val Leu Lys Glu Met His Arg Val Thr Lys
    130                 135                 140
CCG GGC GGC ATA ACC GTC TGC CTG GAA ACG TCG CAG CCG ACG CTG TTC  450
Pro Gly Gly Ile Thr Val Cys Leu Glu Thr Ser Gln Pro Thr Leu Phe
145                 150                 155                 160
GGG TTT CGC CAG CTT TAC TAT TTT TAC TTC CGG TTT ATT ATG CCG CTG  495
Gly Phe Arg Gln Leu Tyr Tyr Phe Tyr Phe Arg Phe Ile Met Pro Leu
                165                 170                 175
TTT GGC AAG CTG CTG GCG AAA AGC TAT GAG GAG TAC TCG TGG CTG CAG  540
Phe Gly Lys Leu Leu Ala Lys Ser Tyr Glu Glu Tyr Ser Trp Leu Gln
            180                 185                 190
GAA TCG GCG CGC GAG TTT CCG GGG CGG GAC GAG CTG GCC GAG ATG TTC  585
Glu Ser Ala Arg Glu Phe Pro Gly Arg Asp Glu Leu Ala Glu Met Phe
        195                 200                 205
CGC GCC GCC GGT TTT GTC GAT GTC GAG GTC AAA CCG TAC ACG TTT GGC  630
Arg Ala Ala Gly Phe Val Asp Val Glu Val Lys Pro Tyr Thr Phe Gly
    210                 215                 220
```

```
GTG GCG GCG ATG CAC TTG GGC TAT AAA CGG TGA                    675
Val Ala Ala Met His Leu Gly Tyr Lys Arg ***
225                 230

                                                               705
```

Sequence No.: 3
Sequence length: 972
Sequence type: nucleic acid
Strandedness: double
Topology: linear
Molecule type: Genomic DNA
Original source:
Organism: *Bacillus stearothermophilus*
Sequence:

```
GTG AAC AAC ATG AAG TTA AAG GCG ATG TAT TCG TTT TTA AGC GAT GAT   45
Val Asn Asn Met Lys Leu Lys Ala Met Tyr Ser Phe Leu Ser Asp Asp
                  5                  10                  15
TTA GCG GCG GTC GAA GAG GAG CTT GAG CGG GCG GTT CAG TCG GAA TAC   90
Leu Ala Ala Val Glu Glu Glu Leu Glu Arg Ala Val Gln Ser Glu Tyr
             20                  25                  30
GGG CCG CTT GGG GAA GCG GCG CTC CAT CTG TTG CAG GCG GGC GGA AAG  135
Gly Pro Leu Gly Glu Ala Ala Leu His Leu Leu Gln Ala Gly Gly Lys
         35                  40                  45
CGG ATC CGT CCC GTT TTT GTC TTG CTT GCC GCC CGC TTC GGC CAA TAT  180
Arg Ile Arg Pro Val Phe Val Leu Leu Ala Ala Arg Phe Gly Gln Tyr
     50                  55                  60
GAC CTT GAG CGG ATG AAG CAT GTT GCC GTT GCG CTC GAG CTC ATT CAT  225
Asp Leu Glu Arg Met Lys His Val Ala Val Ala Leu Glu Leu Ile His
 65                  70                  75                  80
ATG GCT TCG CTC GTC CAC GAC GAT GTG ATC GAC GAC GCC GAT TTG CGC  270
Met Ala Ser Leu Val His Asp Asp Val Ile Asp Asp Ala Asp Leu Arg
                 85                  90                  95
CGC GGC CGG CCG ACG ATC AAG GCG AAA TGG AGC AAC CGG TTC GCC ATG  315
Arg Gly Arg Pro Thr Ile Lys Ala Lys Trp Ser Asn Arg Phe Ala Met
            100                 105                 110
```

```
TAC ACA GGG GAT TAT TTG TTT GCC CGC TCG CTC GAA CGG ATG GCG GAG  360
Tyr Thr Gly Asp Tyr Leu Phe Ala Arg Ser Leu Glu Arg Met Ala Glu
        115                 120                 125
CTC GGC AAC CCG CGC GCC CAT CAA GTG TTG GCG AAA ACG ATC GTG GAA  405
Leu Gly Asn Pro Arg Ala His Gln Val Leu Ala Lys Thr Ile Val Glu
    130                 135                 140
GTG TGC CGC GGG GAA ATT GAG CAA ATT AAA GAC AAG TAC CGG TTT GAT  450
Val Cys Arg Gly Glu Ile Glu Gln Ile Lys Asp Lys Tyr Arg Phe Asp
145                 150                 155                 160
CAG CCG CTG CGC ACG TAT TTG CGG CGC ATC CGT CGG AAA ACG GCG CTG  495
Gln Pro Leu Arg Thr Tyr Leu Arg Arg Ile Arg Arg Lys Thr Ala Leu
                165                 170                 175
CTC ATC GCC GCG AGC TGC CAG CTT GGC GCC CTC GCT GCC GGC GCG CCG  540
Leu Ile Ala Ala Ser Cys Gln Leu Gly Ala Leu Ala Ala Gly Ala Pro
            180                 185                 190
GAG CCG ATT GTG AAG CGG CTG TAC TGG TTC GGC CAT TAT GTC GGC ATG  585
Glu Pro Ile Val Lys Arg Leu Tyr Trp Phe Gly His Tyr Val Gly Met
        195                 200                 205
TCG TTT CAA ATT ACC GAC GAC ATT CTC GAT TTC ACT GGG ACG GAG GAA  630
Ser Phe Gln Ile Thr Asp Asp Ile Leu Asp Phe Thr Gly Thr Glu Glu
    210                 215                 220
CAG CTC GGC AAA CCG GCC GGA AGC GAC TTG CTA CAA GGA AAC GTC ACC  675
Gln Leu Gly Lys Pro Ala Gly Ser Asp Leu Leu Gln Gly Asn Val Thr
225                 230                 235                 240
CTT CCT GTG CTG TAT GCC TTG AGC GAT GAG CGG GTG AAG GCG GCC ATT  720
Leu Pro Val Leu Tyr Ala Leu Ser Asp Glu Arg Val Lys Ala Ala Ile
                245                 250                 255
GCA GCT GTC GGT CCG GAA ACG GAC GTT GCG GAA ATG GCG GCG GTC ATT  765
Ala Ala Val Gly Pro Glu Thr Asp Val Ala Glu Met Ala Ala Val Ile
            260                 265                 270
TCC GCC ATT AAG CGG ACG GAC GCC ATT GAG CGG TCG TAT GCG TTA AGC  810
Ser Ala Ile Lys Arg Thr Asp Ala Ile Glu Arg Ser Tyr Ala Leu Ser
        275                 280                 285
GAC CGT TAC CTT GAC AAG GCG CTT CAC CTT CTT GAC GGA CTG CCG ATG  855
Asp Arg Tyr Leu Asp Lys Ala Leu His Leu Leu Asp Gly Leu Pro Met
    290                 295                 300
```

```
AAT GAG GCG CGC GGC CTG TTG CGC GAC CTC GCC CTT TAC ATC GGG AAA  900
Asn Glu Ala Arg Gly Leu Leu Arg Asp Leu Ala Leu Tyr Ile Gly Lys
305                 310                 315                 320
AGG GAT TAT TAA                                              945
Arg Asp Tyr ***
                                                             972
```

Sequence No.: 4
Sequence length: 30
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:

```
CTNATHCAYG AYGAYYTNCC NTCNATGGAC                             30
```

Sequence No.: 5
Sequence length: 24
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:

```
GAYAAYGAYG AYYTNMGNMG NGGC                                   24
```

Sequence No.: 6
Sequence length: 27
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:

```
ATCRTCNCKD ATYTGRAANG CNARNCC                                27
```

Sequence No.: 7
Sequence length: 27
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:

ATCNARDATR TCRTCNCKDA TYTGRAA 27

Sequence No.: 8
Sequence length: 21
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
GTCRCTNCCN ACNGGYTTNC C 21

Sequence No.: 9
Sequence length: 20
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
YTNGARGCNG GNGGNAARMG 20

Sequence No.: 10
Sequence length: 20
Sequence type:
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
TAYWSNYTNA THCAYGAYGA 20

Sequence No.: 11
Sequence length: 21
Sequence type:
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
YTCCATRTCN GCNGCYTGNC C 21

Sequence No.: 12
Sequence length: 26
Sequence type: nucleic acid
Strandedness: single

Topology: linear
Molecule type: Synthetic DNA
Sequence:
YTNGARTAYA THCAYMGNCA YAARAC 26

Sequence No.: 13
Sequence length: 18
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
DATRTCNARD ATRTCRTC 18

Sequence No.: 14
Sequence length: 20
Sequence type: nucleic acid
Strandedness: single
Topology: linear
Molecule type: Synthetic DNA
Sequence:
GATCACATCG TCGTGGACGA 20

Heptaprenyl diphosphate (HDP)-synthetase derived from *Bacillus stearothermophilus* which enzymes have the amino acid sequences shown as SEQ ID NOs: 1 to 3; 1 and 2; 2 and 3; or 1 and 3, DNA encoding them, and a method of producing the enzymes.

According to the invention it is possible to industrially produce HDP-synthesizing enzyme and HPD.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Toyota Jidosha Kabushiki Kaisha
(B) STREET: 1, Toyota-cho
(C) CITY: Toyota-shi
(D) STATE: Aichi
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): None

(ii) TITLE OF INVENTION: HEPTAPRENYL DIPHOSPHATE-SYNTHESIZING ENZYME AND DNA ENCODING SAME

(iii) NUMBER OF SEQUENCES: 17

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 95111764.7

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 663 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..663

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG CTC GAT GGC GCT TCA ACG GCG CCG AGT GAG GCG GAG CGG TGC ATC        48
Met Leu Asp Gly Ala Ser Thr Ala Pro Ser Glu Ala Glu Arg Cys Ile
 1               5                  10                  15

ATC GCC ATG ATG CTC ATG CAG ATC GCC CTT GAT ACC CAC GAT GAG GTG        96
Ile Ala Met Met Leu Met Gln Ile Ala Leu Asp Thr His Asp Glu Val
            20                  25                  30
```

```
ACA GAT GAC GGC GGC GAC TTG CGG GCG CGG CAG CTT GTC GTC CTG GCC        144
Thr Asp Asp Gly Gly Asp Leu Arg Ala Arg Gln Leu Val Val Leu Ala
          35                  40                  45

GGC GAC TTG TAC AGC GGG CTG TAC TAT GAG TTG TTG GCG CGT TCG GGC        192
Gly Asp Leu Tyr Ser Gly Leu Tyr Tyr Glu Leu Leu Ala Arg Ser Gly
      50                  55                  60

GAA ACG GCG CTC ATC CGC TCG TTC GCC GAG GCG GTC CGC GAT ATT AAC        240
Glu Thr Ala Leu Ile Arg Ser Phe Ala Glu Ala Val Arg Asp Ile Asn
 65                  70                  75                  80

GAG CAA AAA GTG CGG CTT TAC GAA AAA AAA GTA GAG CGG ATC GAG TCG        288
Glu Gln Lys Val Arg Leu Tyr Glu Lys Lys Val Glu Arg Ile Glu Ser
                 85                  90                  95

TTG TTT GCG GCG GTC GGC ACG ATC GAA TCG GCG TTG CTT GTC AAG CTC        336
Leu Phe Ala Ala Val Gly Thr Ile Glu Ser Ala Leu Leu Val Lys Leu
            100                 105                 110

GCC GAC CGC ATG GCG GCG CCG CAG TGG GGG CAG TTT GCC TAT TCG TAT        384
Ala Asp Arg Met Ala Ala Pro Gln Trp Gly Gln Phe Ala Tyr Ser Tyr
        115                 120                 125

TTG CTG ATG CGG CGC CTG CTG CTC GAG CAG GAA GCG TTC ATC CGC ACG        432
Leu Leu Met Arg Arg Leu Leu Leu Glu Gln Glu Ala Phe Ile Arg Thr
    130                 135                 140

GGA GCT TCG GTG CTC TTT GAG CAA ATG GCG CAA ATC GCG TTC CCG CGC        480
Gly Ala Ser Val Leu Phe Glu Gln Met Ala Gln Ile Ala Phe Pro Arg
145                 150                 155                 160

GCG GAA ACG TTG ACG AAA GAG CAA AAG CGG CAT TTG CTC CGC TTT TGC        528
Ala Glu Thr Leu Thr Lys Glu Gln Lys Arg His Leu Leu Arg Phe Cys
                165                 170                 175

CGC CGC TAT ATC GAC GGC TGC CGG GAG GCG CTG TTT GCG GCG AAA CTG        576
Arg Arg Tyr Ile Asp Gly Cys Arg Glu Ala Leu Phe Ala Ala Lys Leu
            180                 185                 190

CCG GTC AAC GGC CTG CTG CAG CTC CGC GTG GCC GTG CTT TCC GGC GGG        624
Pro Val Asn Gly Leu Leu Gln Leu Arg Val Ala Val Leu Ser Gly Gly
        195                 200                 205

TTT CAA GCC ATC GCC AAA AAG ACG GTG GAA GAA GGG TAG                    663
Phe Gln Ala Ile Ala Lys Lys Thr Val Glu Glu Gly
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 220 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Leu Asp Gly Ala Ser Thr Ala Pro Ser Glu Ala Glu Arg Cys Ile
 1               5                  10                  15

Ile Ala Met Met Leu Met Gln Ile Ala Leu Asp Thr His Asp Glu Val
            20                  25                  30

Thr Asp Asp Gly Gly Asp Leu Arg Ala Arg Gln Leu Val Val Leu Ala
        35                  40                  45

Gly Asp Leu Tyr Ser Gly Leu Tyr Tyr Glu Leu Leu Ala Arg Ser Gly
    50                  55                  60

Glu Thr Ala Leu Ile Arg Ser Phe Ala Glu Ala Val Arg Asp Ile Asn
65                  70                  75                  80

Glu Gln Lys Val Arg Leu Tyr Glu Lys Lys Val Glu Arg Ile Glu Ser
                85                  90                  95

Leu Phe Ala Ala Val Gly Thr Ile Glu Ser Ala Leu Leu Val Lys Leu
            100                 105                 110

Ala Asp Arg Met Ala Ala Pro Gln Trp Gly Gln Phe Ala Tyr Ser Tyr
        115                 120                 125

Leu Leu Met Arg Arg Leu Leu Leu Glu Gln Glu Ala Phe Ile Arg Thr
    130                 135                 140

Gly Ala Ser Val Leu Phe Glu Gln Met Ala Gln Ile Ala Phe Pro Arg
145                 150                 155                 160

Ala Glu Thr Leu Thr Lys Glu Gln Lys Arg His Leu Leu Arg Phe Cys
                165                 170                 175

Arg Arg Tyr Ile Asp Gly Cys Arg Glu Ala Leu Phe Ala Ala Lys Leu
            180                 185                 190

Pro Val Asn Gly Leu Leu Gln Leu Arg Val Ala Val Leu Ser Gly Gly
        195                 200                 205

Phe Gln Ala Ile Ala Lys Lys Thr Val Glu Glu Gly
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 705 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..705

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
ATG CGT CAA TCG AAA GAA GAG CGA GTC CAT CGC GTA TTT GAA AAC ATT      48
Met Arg Gln Ser Lys Glu Glu Arg Val His Arg Val Phe Glu Asn Ile
 1               5                  10                  15

TCT GCG CAT TAT GAC CGG ATG AAC TCC GTC ATC AGC TTC CGC CGC CAC      96
Ser Ala His Tyr Asp Arg Met Asn Ser Val Ile Ser Phe Arg Arg His
            20                  25                  30

TTG AAG TGG CGC AAA GAC GTG ATG CGG CGG ATG AAT GTG CAA AAA GGC     144
Leu Lys Trp Arg Lys Asp Val Met Arg Arg Met Asn Val Gln Lys Gly
        35                  40                  45

AAA AAA GCG CTC GAT GTG TGC TGT GGG ACG GCT GAC TGG ACG ATC GCC     192
Lys Lys Ala Leu Asp Val Cys Cys Gly Thr Ala Asp Trp Thr Ile Ala
    50                  55                  60

TTG GCG GAG GCG GTC GGT CCG GAA GGG AAA GTG TAC GGC CTT GAT TTC     240
Leu Ala Glu Ala Val Gly Pro Glu Gly Lys Val Tyr Gly Leu Asp Phe
65                  70                  75                  80

AGC GAA AAC ATG CTG AAA GTC GGC GAA CAG AAG GTA AAA GCG CGC GGG     288
Ser Glu Asn Met Leu Lys Val Gly Glu Gln Lys Val Lys Ala Arg Gly
                85                  90                  95

TTG CAT AAT GTG AAG CTC ATT CAC GGC AAT GCG ATG CAG CTG CCG TTT     336
Leu His Asn Val Lys Leu Ile His Gly Asn Ala Met Gln Leu Pro Phe
            100                 105                 110

CCT GAC AAT TCG TTC GAT TAT GTG ACG ATC GGC TTC GGT TTG CGC AAC     384
Pro Asp Asn Ser Phe Asp Tyr Val Thr Ile Gly Phe Gly Leu Arg Asn
        115                 120                 125

GTC CCT GAC TAT ATG ACC GTG CTT AAG GAA ATG CAC CGG GTG ACG AAG     432
Val Pro Asp Tyr Met Thr Val Leu Lys Glu Met His Arg Val Thr Lys
    130                 135                 140
```

```
CCG GGC GGC ATA ACC GTC TGC CTG GAA ACG TCG CAG CCG ACG CTG TTC        480
Pro Gly Gly Ile Thr Val Cys Leu Glu Thr Ser Gln Pro Thr Leu Phe
145                 150                 155                 160

GGG TTT CGC CAG CTT TAC TAT TTT TAC TTC CGG TTT ATT ATG CCG CTG        528
Gly Phe Arg Gln Leu Tyr Tyr Phe Tyr Phe Arg Phe Ile Met Pro Leu
                165                 170                 175

TTT GGC AAG CTG CTG GCG AAA AGC TAT GAG GAG TAC TCG TGG CTG CAG        576
Phe Gly Lys Leu Leu Ala Lys Ser Tyr Glu Glu Tyr Ser Trp Leu Gln
            180                 185                 190

GAA TCG GCG CGC GAG TTT CCG GGG CGG GAC GAG CTG GCC GAG ATG TTC        624
Glu Ser Ala Arg Glu Phe Pro Gly Arg Asp Glu Leu Ala Glu Met Phe
        195                 200                 205

CGC GCC GCC GGT TTT GTC GAT GTC GAG GTC AAA CCG TAC ACG TTT GGC        672
Arg Ala Ala Gly Phe Val Asp Val Glu Val Lys Pro Tyr Thr Phe Gly
    210                 215                 220

GTG GCG GCG ATG CAC TTG GGC TAT AAA CGG TGA                            705
Val Ala Ala Met His Leu Gly Tyr Lys Arg
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 234 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Arg Gln Ser Lys Glu Glu Arg Val His Arg Val Phe Glu Asn Ile
 1               5                  10                  15

Ser Ala His Tyr Asp Arg Met Asn Ser Val Ile Ser Phe Arg Arg His
            20                  25                  30

Leu Lys Trp Arg Lys Asp Val Met Arg Arg Met Asn Val Gln Lys Gly
        35                  40                  45

Lys Lys Ala Leu Asp Val Cys Cys Gly Thr Ala Asp Trp Thr Ile Ala
    50                  55                  60

Leu Ala Glu Ala Val Gly Pro Glu Gly Lys Val Tyr Gly Leu Asp Phe
65                  70                  75                  80

Ser Glu Asn Met Leu Lys Val Gly Glu Gln Lys Val Lys Ala Arg Gly
                85                  90                  95

Leu His Asn Val Lys Leu Ile His Gly Asn Ala Met Gln Leu Pro Phe
            100                 105                 110

Pro Asp Asn Ser Phe Asp Tyr Val Thr Ile Gly Phe Gly Leu Arg Asn
        115                 120                 125

Val Pro Asp Tyr Met Thr Val Leu Lys Glu Met His Arg Val Thr Lys
    130                 135                 140

Pro Gly Gly Ile Thr Val Cys Leu Glu Thr Ser Gln Pro Thr Leu Phe
145                 150                 155                 160

Gly Phe Arg Gln Leu Tyr Tyr Phe Tyr Phe Arg Phe Ile Met Pro Leu
                165                 170                 175

Phe Gly Lys Leu Leu Ala Lys Ser Tyr Glu Glu Tyr Ser Trp Leu Gln
            180                 185                 190

Glu Ser Ala Arg Glu Phe Pro Gly Arg Asp Glu Leu Ala Glu Met Phe
        195                 200                 205

Arg Ala Ala Gly Phe Val Asp Val Glu Val Lys Pro Tyr Thr Phe Gly
    210                 215                 220

Val Ala Ala Met His Leu Gly Tyr Lys Arg
225                 230
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 972 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..972

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
GTG AAC AAC ATG AAG TTA AAG GCG ATG TAT TCG TTT TTA AGC GAT GAT      48
Val Asn Asn Met Lys Leu Lys Ala Met Tyr Ser Phe Leu Ser Asp Asp
 1               5                  10                  15

TTA GCG GCG GTC GAA GAG GAG CTT GAG CGG GCG GTT CAG TCG GAA TAC      96
Leu Ala Ala Val Glu Glu Glu Leu Glu Arg Ala Val Gln Ser Glu Tyr
            20                  25                  30

GGG CCG CTT GGG GAA GCG GCG CTC CAT CTG TTG CAG GCG GGC GGA AAG     144
Gly Pro Leu Gly Glu Ala Ala Leu His Leu Leu Gln Ala Gly Gly Lys
        35                  40                  45

CGG ATC CGT CCC GTT TTT GTC TTG CTT GCC GCC CGC TTC GGC CAA TAT     192
Arg Ile Arg Pro Val Phe Val Leu Leu Ala Ala Arg Phe Gly Gln Tyr
    50                  55                  60

GAC CTT GAG CGG ATG AAG CAT GTT GCC GTT GCG CTC GAG CTC ATT CAT     240
Asp Leu Glu Arg Met Lys His Val Ala Val Ala Leu Glu Leu Ile His
65                  70                  75                  80

ATG GCT TCG CTC GTC CAC GAC GAT GTG ATC GAC GAC GCC GAT TTG CGC     288
Met Ala Ser Leu Val His Asp Asp Val Ile Asp Asp Ala Asp Leu Arg
                85                  90                  95

CGC GGC CGG CCG ACG ATC AAG GCG AAA TGG AGC AAC CGG TTC GCC ATG     336
Arg Gly Arg Pro Thr Ile Lys Ala Lys Trp Ser Asn Arg Phe Ala Met
            100                 105                 110

TAC ACA GGG GAT TAT TTG TTT GCC CGC TCG CTC GAA CGG ATG GCG GAG     384
Tyr Thr Gly Asp Tyr Leu Phe Ala Arg Ser Leu Glu Arg Met Ala Glu
        115                 120                 125

CTC GGC AAC CCG CGC GCC CAT CAA GTG TTG GCG AAA ACG ATC GTG GAA     432
Leu Gly Asn Pro Arg Ala His Gln Val Leu Ala Lys Thr Ile Val Glu
    130                 135                 140
```

```
GTG TGC CGC GGG GAA ATT GAG CAA ATT AAA GAC AAG TAC CGG TTT GAT      480
Val Cys Arg Gly Glu Ile Glu Gln Ile Lys Asp Lys Tyr Arg Phe Asp
145                 150                 155                 160

CAG CCG CTG CGC ACG TAT TTG CGG CGC ATC CGT CGG AAA ACG GCG CTG      528
Gln Pro Leu Arg Thr Tyr Leu Arg Arg Ile Arg Arg Lys Thr Ala Leu
                165                 170                 175

CTC ATC GCC GCG AGC TGC CAG CTT GGC GCC CTC GCT GCC GGC GCG CCG      576
Leu Ile Ala Ala Ser Cys Gln Leu Gly Ala Leu Ala Ala Gly Ala Pro
            180                 185                 190

GAG CCG ATT GTG AAG CGG CTG TAC TGG TTC GGC CAT TAT GTC GGC ATG      624
Glu Pro Ile Val Lys Arg Leu Tyr Trp Phe Gly His Tyr Val Gly Met
        195                 200                 205

TCG TTT CAA ATT ACC GAC GAC ATT CTC GAT TTC ACT GGG ACG GAG GAA      672
Ser Phe Gln Ile Thr Asp Asp Ile Leu Asp Phe Thr Gly Thr Glu Glu
    210                 215                 220

CAG CTC GGC AAA CCG GCC GGA AGC GAC TTG CTA CAA GGA AAC GTC ACC      720
Gln Leu Gly Lys Pro Ala Gly Ser Asp Leu Leu Gln Gly Asn Val Thr
225                 230                 235                 240

CTT CCT GTG CTG TAT GCC TTG AGC GAT GAG CGG GTG AAG GCG GCC ATT      768
Leu Pro Val Leu Tyr Ala Leu Ser Asp Glu Arg Val Lys Ala Ala Ile
                245                 250                 255

GCA GCT GTC GGT CCG GAA ACG GAC GTT GCG GAA ATG GCG GCG GTC ATT      816
Ala Ala Val Gly Pro Glu Thr Asp Val Ala Glu Met Ala Ala Val Ile
            260                 265                 270

TCC GCC ATT AAG CGG ACG GAC GCC ATT GAG CGG TCG TAT GCG TTA AGC      864
Ser Ala Ile Lys Arg Thr Asp Ala Ile Glu Arg Ser Tyr Ala Leu Ser
        275                 280                 285

GAC CGT TAC CTT GAC AAG GCG CTT CAC CTT CTT GAC GGA CTG CCG ATG      912
Asp Arg Tyr Leu Asp Lys Ala Leu His Leu Leu Asp Gly Leu Pro Met
    290                 295                 300

AAT GAG GCG CGC GGC CTG TTG CGC GAC CTC GCC CTT TAC ATC GGG AAA      960
Asn Glu Ala Arg Gly Leu Leu Arg Asp Leu Ala Leu Tyr Ile Gly Lys
305                 310                 315                 320

AGG GAT TAT TAA                                                     972
Arg Asp Tyr
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 323 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Val Asn Asn Met Lys Leu Lys Ala Met Tyr Ser Phe Leu Ser Asp Asp
 1               5                  10                  15

Leu Ala Ala Val Glu Glu Glu Leu Glu Arg Ala Val Gln Ser Glu Tyr
            20                  25                  30

Gly Pro Leu Gly Glu Ala Ala Leu His Leu Leu Gln Ala Gly Gly Lys
        35                  40                  45

Arg Ile Arg Pro Val Phe Val Leu Leu Ala Ala Arg Phe Gly Gln Tyr
    50                  55                  60

Asp Leu Glu Arg Met Lys His Val Ala Val Ala Leu Glu Leu Ile His
65                  70                  75                  80

Met Ala Ser Leu Val His Asp Asp Val Ile Asp Asp Ala Asp Leu Arg
                85                  90                  95

Arg Gly Arg Pro Thr Ile Lys Ala Lys Trp Ser Asn Arg Phe Ala Met
            100                 105                 110

Tyr Thr Gly Asp Tyr Leu Phe Ala Arg Ser Leu Glu Arg Met Ala Glu
        115                 120                 125

Leu Gly Asn Pro Arg Ala His Gln Val Leu Ala Lys Thr Ile Val Glu
    130                 135                 140

Val Cys Arg Gly Glu Ile Glu Gln Ile Lys Asp Lys Tyr Arg Phe Asp
145                 150                 155                 160

Gln Pro Leu Arg Thr Tyr Leu Arg Arg Ile Arg Arg Lys Thr Ala Leu
                165                 170                 175

Leu Ile Ala Ala Ser Cys Gln Leu Gly Ala Leu Ala Ala Gly Ala Pro
            180                 185                 190

Glu Pro Ile Val Lys Arg Leu Tyr Trp Phe Gly His Tyr Val Gly Met
        195                 200                 205

Ser Phe Gln Ile Thr Asp Asp Ile Leu Asp Phe Thr Gly Thr Glu Glu
    210                 215                 220

Gln Leu Gly Lys Pro Ala Gly Ser Asp Leu Leu Gln Gly Asn Val Thr
225                 230                 235                 240

Leu Pro Val Leu Tyr Ala Leu Ser Asp Glu Arg Val Lys Ala Ala Ile
                245                 250                 255
```

```
Ala Ala Val Gly Pro Glu Thr Asp Val Ala Glu Met Ala Ala Val Ile
            260                 265                 270

Ser Ala Ile Lys Arg Thr Asp Ala Ile Glu Arg Ser Tyr Ala Leu Ser
        275                 280                 285

Asp Arg Tyr Leu Asp Lys Ala Leu His Leu Leu Asp Gly Leu Pro Met
    290                 295                 300

Asn Glu Ala Arg Gly Leu Leu Arg Asp Leu Ala Leu Tyr Ile Gly Lys
305                 310                 315                 320

Arg Asp Tyr
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTNATHCAYG AYGAYYTNCC NTCNATGGAC 30

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GAYAAYGAYG AYYTNMGNMG NGGC 24

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ATCRTCNCKD ATYTGRAANG CNARNCC 27

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATCNARDATR TCRTCNCKDA TYTGRAA 27

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTCRCTNCCN ACNGGYTTNC C 21

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

YTNGARGCNG GNGGNAARMG 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TAYWSNYTNA THCAYGAYGA 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

YTCCATRTCN GCNGCYTGNC C 21

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

YTNGARTAYA THCAYMGNCA YAARAC 26

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

DATRTCNARD ATRTCRTC 18

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GATCACATCG TCGTGGACGA 20

Claims

1. A protein of *Bacillus stearothermophilus* origin with heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; a peptide with the amino acid sequence from the 1st amino acid Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

2. A peptide of *Bacillus stearothermophilus* origin which has the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

3. A peptide of *Bacillus stearothermophilus* origin, which has the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

4. A protein of *Bacillus stearothermophilus* origin with heptaprenyl diphosphate synthetase activity, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

5. A protein of *Bacillus stearothermophilus* origin, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 220th amino acid Gly of Sequence No. 1, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

6. A protein of *Bacillus stearothermophilus* origin, which comprises a peptide with the amino acid sequence from the 1st amino acid Met to the 234th amino acid Arg of Sequence No. 2, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence; and a peptide with the amino acid sequence from the 1st amino acid Val to the 323rd amino acid Tyr of Sequence No. 3, or an amino acid sequence resulting from a substitution, deletion or addition of one or a few amino acids in the amino acid sequence.

7. DNA containing a base sequence encoding the 3 peptides according to Claim 1.

8. DNA encoding the peptide according to Claim 2.

9. DNA encoding the peptide according to Claim 3.

10. DNA encoding the two peptides according to Claim 4.

11. DNA encoding the two peptides according to Claim 5.

12. DNA encoding the two peptides according to Claim 6.

13. An expression vector comprising the DNA according to Claim 7.

14. A host transformed by the expression vector according to Claim 13.

15. The host according to Claim 14 which is a bacterium.

16. The host according to Claim 15 which is *Escherichia*.

17. A method of producing a peptide with heptaprenyl diphosphate synthetase activity or a related peptide, comprising the steps of culturing a host according to Claim 14, and recovering from the culture a peptide with heptaprenyl diphosphate synthetase activity or a related peptide.

18. A method of producing heptaprenyl diphosphate, comprising the steps of culturing a host according to Claim 14, and recovering heptaprenyl diphosphate from the culture.

19. A method of producing heptaprenyl diphosphate, comprising the steps of allowing the heptaprenyl diphosphate-synthesizing enzyme according to Claim 1, or a substance containing it, to act on an isopentenyl diphosphate, farnesyl diphosphate, geranylgeranyl diphosphate, farnesylgeranyl diphosphate or hexaprenyl diphosphate substrate.

Fig.1

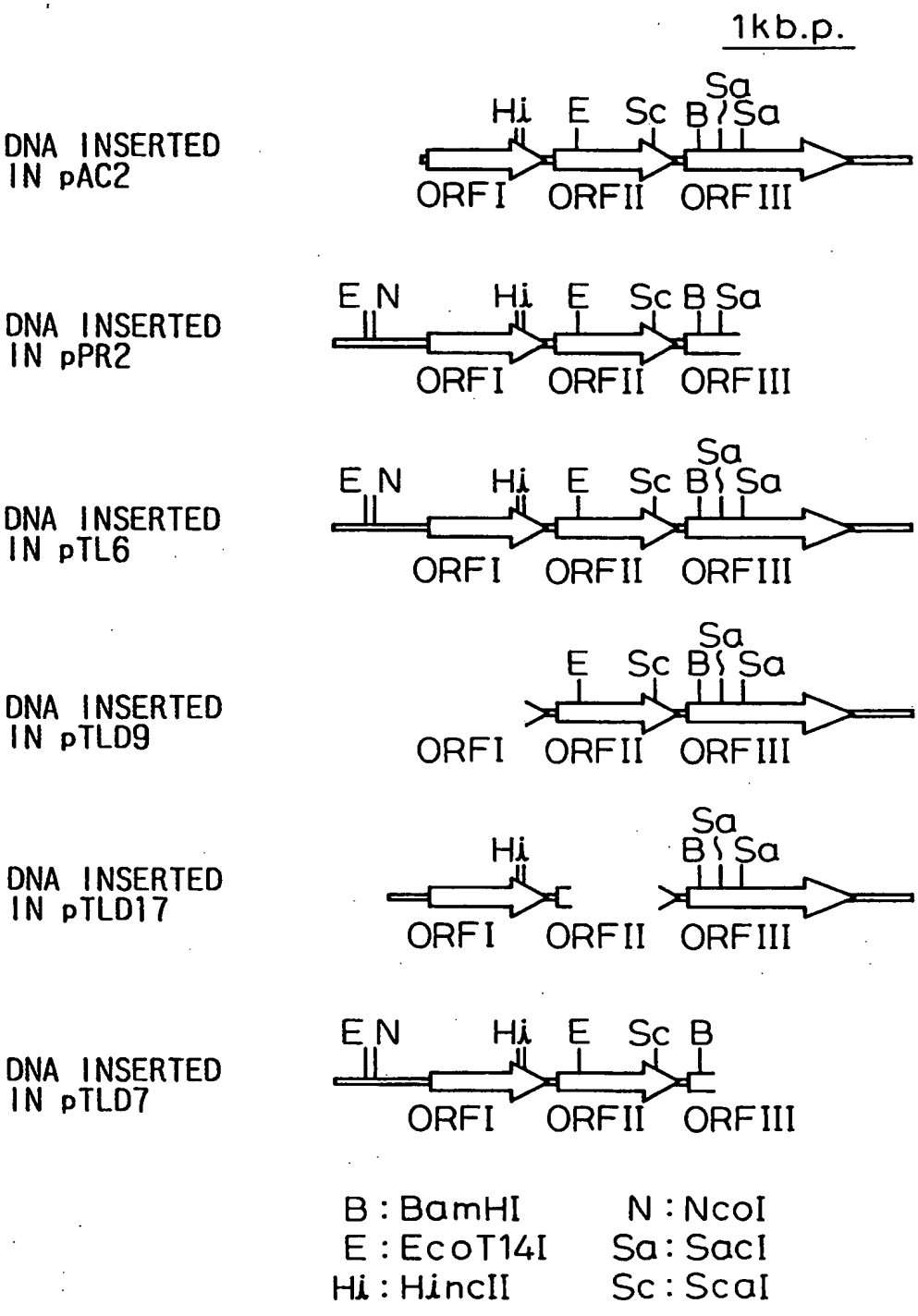
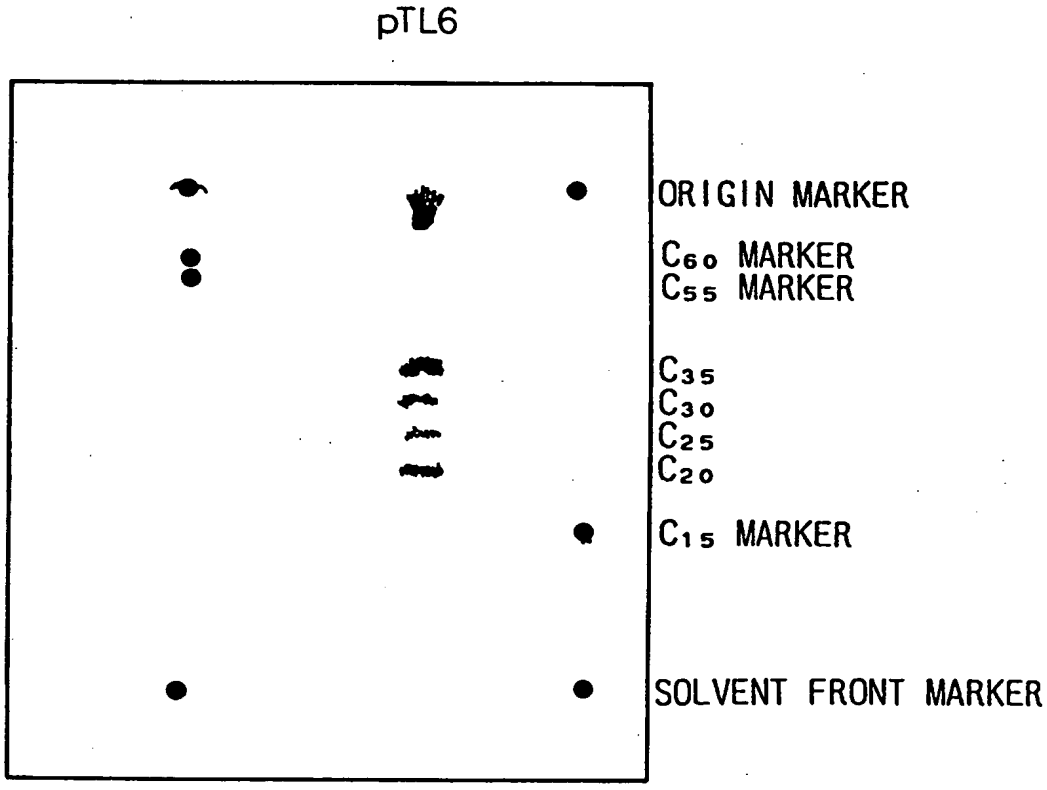

1kb.p.
DNA INSERTED IN pAC2
Hi E Sc B Sa
ORFI ORFII ORFIII
DNA INSERTED IN pPR2
E N Hi E Sc B Sa
ORFI ORFII ORFIII
DNA INSERTED IN pTL6
E N Hi E Sc B Sa
ORFI ORFII ORFIII
DNA INSERTED IN pTLD9
E Sc B Sa
ORFI ORFII ORFIII
DNA INSERTED IN pTLD17
Hi B Sa
ORFI ORFII ORFIII
DNA INSERTED IN pTLD7
E N Hi E Sc B
ORFI ORFII ORFIII
B : BamHI
E : EcoT14I
Hi : HincII
N : NcoI
Sa : SacI
Sc : ScaI

Fig.2


pTL6
ORIGIN MARKER
C60 MARKER
C55 MARKER
C35
C30
C25
C20
C15 MARKER
SOLVENT FRONT MARKER